Quantification of T-cell dynamics

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What is the difficulty?

How to follow a lymphocyte from its birth to its death?

Extrapolation from mice to men

How long does a T cell live?

• Estimates vary widely

• How is T-cell turnover disturbed in HIV infection, leukemia, or after stem cell transplantation?

Experiments of nature (1):
T-cell reconstitution after chemotherapy

Underestimate?: Cells may also die during reconstitution
Overestimate?: Cells undergo little competition

Mackall et al. 1995
Experiments of nature (2):
Loss of T cells with chromosome damage

Immunological memory resides in a population with rapid turnover:
Memory T cells have a shorter lifespan (~250 d) than naïve T cells (~1000 d)

Static versus dynamic markers of T-lymphocyte turnover

Static
- Ki67-expression
  (protein expressed in G1,S,G2,M phase)
- Annexin V staining
  (stains phosphatidylserine translocation)

Dynamic
- Natural markers:
  - T-cell telomere lengths
  - T-cell receptor excision circles

Labelling:
- (CFSE labelling)
- BrdU labelling
- Stable isotope labelling

Changes in telomere lengths are no direct measure of T-cell division

Changes in TRECs do not directly reflect thymus output

Caution: Cells have DNA damage, and cell numbers are low

Michie et al. Nature 1992

Weng et al. PNAS 1995

Healthy individuals

Thymectomized individuals

HIV-infected individuals

Static versus dynamic markers of T-lymphocyte turnover

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Labelling:
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Determine percentage BrdU+ cells by FACS analysis

Kovacs et al. 2001

How to quantify leukocyte turnover?

Dynamic markers (1)
BrdU Labelling

BrdU = 5-bromo-2'-deoxyuridine
Nucleoside analog incorporated instead of thymidine

During label administration:
Production (at rate p):
U → 2L
L → 2L
Death removes cells at rate d

After stop of label:
Production (at rate p):
L → 2L
U → 2U
Death removes cells at rate d

U+L=1
### Model for BrdU labelling

\[
\frac{dL}{dt} = s + 2pU + pL - dL \\
\frac{dU}{dt} = -pU - dU
\]

- **L**: up-labelling
- **U**: down-labelling

\[
\frac{dL}{dt} = s + pU - dU \\
\frac{dU}{dt} = pL - dL
\]

- **L=1-U**: determined by \(p+d\)

### Expected changes in the percentage of BrdU+ cells

Kovacs et al. 2001

See today’s exercise

But… possible toxicity, almost only done in mice, only short-term labelling

### Dynamic markers (2)

#### Stable isotope labelling

- **Deuterium (\(^2\)H) as \(^2\)H\(_2\)O or \(^2\)H-glucose**
- **Non-toxic and no interference with cell dynamics**
- **de novo nucleotide synthesis pathway**
  - \(^2\)H is incorporated into DNA of newly dividing cells

- **Fraction of labeled DNA** is measured by GC-MS

### Stable isotope labelling

- **\(^2\)H-glucose**
- **\(^2\)H\(_2\)O**

- **Intravenously**
  - Hellerstein et al. 1999, 2003
  - McCune et al. 2000
  - Mohri et al. 2001
  - Ribeiro et al. 2002
  - Macallan et al. 2003, 2004
  - Wallace et al. 2004

- **Long-term administration**
  - Hellerstein et al. 2003
  - Vraaeko et al. 2008
During label administration:

Production (at rate p):
- \( L \rightarrow 2L \)
- \( U \rightarrow U+L \)

\( \frac{dL}{dt} = p(L+U) - dL \)

Death removes L at rate d

After stop of label:

Death removes L at rate d

\( \frac{dL}{dt} = -dL \)

Paradox: \( d \) is typically larger than \( p \)

Conclusion: \( d \) is no good measure of average turnover rate

It represents the turnover of \textit{labelled} cells

\(^2\text{H}_2\text{O}\) labeling does not distinguish between production in thymus and periphery!
Average turnover ($p$) needs data during up-labeling period

D-glucose  

D2O

Advantages of heavy water: long-term labeling possible  
many data points during labeling

BrdU
Potentially toxic  
Measures labeled cells
Up: $p+d$  
Down: $p-d$

Deuterium labeling  
Non-toxic (non-radioactive)  
Measures labeled DNA strands

Similarities mouse and human T-cell dynamics

- Small fraction of naive T cells proliferate
- Thymic output declines with age

Steinmann et al. 1985
“Mouse immunology consensus”

- Naive T-cell pool is very dynamic
- New naive T cells come from the thymus

Influence on intuition in human immunology:
- e.g. Loss of CD4 T cells in HIV due to loss of thymic output?

D2O labeling experiment

- Subjects drink $^2$H$_2$O for 9 weeks
- Label enrichment in DNA of studied populations are followed during and after label administration
  - follow-up of 25 weeks in total

T-cell turnover in young healthy men

Labeled naive T cells present 3 years after stop of label

- Labeled naive T cells are very long-lived in humans
In mice, naive T cells live much shorter

Expected lifespan:
- Naive CD4: 6 weeks
- Naive CD8: 11 weeks

Scaling from mice to men...
- Mice live 80 weeks
  - naive CD4 T cells 6 weeks, CD8 11 weeks
- Humans live 80 years
  - naive CD4 T cells 6 years, CD8 10 years

“Mouse immunology consensus”

Naive T-cell pool is highly dynamic
New naive T cells come from the thymus

What is the contribution of thymic output?

Naive T-cell production = Thymic output + T-cell proliferation
Naive T-cell dynamics after thymectomy

Contribution of thymic output and T-cell proliferation in mice

Evidence for naive T-cell proliferation from T-cell receptor excision circles (TRECs)

This is completely different in humans!

Evidence for naive T-cell proliferation in men...
If naive T-cells proliferate homeostatically, TREC contents do decline.

Naive TREC decline in humans suggests that naive T-cell proliferation contributes to the naive T-cell pool in humans.

Hazenberg et al. 2000, Dutile et al. 2003

In human adults <10% of naive T cells is produced by the thymus.

Which part of T-cell production comes from the thymus?

T cells: \[
\frac{dN}{dt} = \sigma(t) + pN - dN
\]

TRECs: \[
\frac{dT}{dt} = e\sigma(t) - dT
\]

TREC content: \[
\frac{dA}{dt} = \frac{\sigma(t)(c - A)}{N} - pA
\]

\[ A/c = \frac{\sigma(t)}{pN + \sigma(t)} \]

Based on CD31

Based on TREC contents

Naive T-cell pool is highly dynamic

New naive T cells come from the thymus

“Mouse immunology consensus”
Naive T-cell maintenance:
a mouse-man divide

Naive T cells in humans are long-lived;
Thymus in human adults plays “little role”

Prediction:
without proliferation there should be no TREC dilution in mice…

And indeed... no TREC decline in naive T cells from mice

“The best-laid schemes o’mice an’ men,
Gang aft agley”

The most carefully prepared plans
may go wrong